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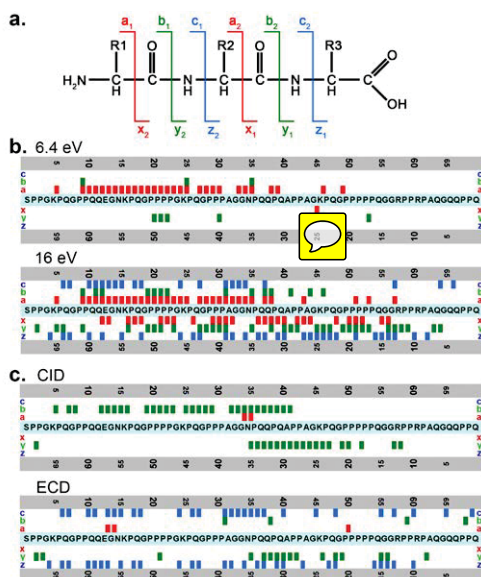
Synchrotron radiation - tandem mass spectrometry for proteomic and structural biology

We introduce a method based upon the coupling of mass spectrometry and VUV-synchrotron radiation for biological investigations. This new approach provides information both on the primary structure of full proteins and on the localization of their noncovalent binding sites. This new methodology has been developed through the study of a human intrinsically disordered protein, namely IB5, and its noncovalent interactions with the tannin procyanidin B2 3'OG.

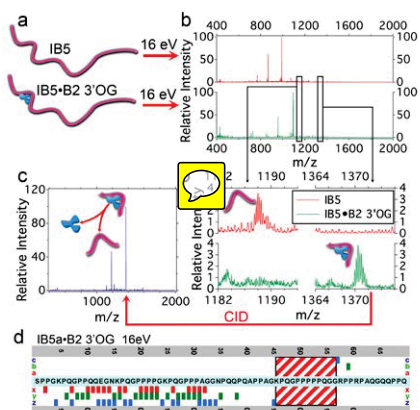
An important part of the proteome is composed of functional proteins that lack ordered structure, under physiological conditions [1]. The peculiar structures and conformations of these proteins, referred to as intrinsically disordered protein (IDP), enable them to fulfill an important repertoire of vital biological functions. Some IDPs have the ability to bind to different and/or several partners, thus producing protein-ligand complexes with distributions of stoichiometries and conformations [2]. Investigations on such systems by classical high-resolution structural methods remain extremely challenging if not intractable, owing to the flexibility and the heterogeneity of the coexisting objects. In contrast, the unique ability offered by mass spectrometry to manipulate *m/z* resolved species reveals a clear advantage when dealing with such biological objects [2]. Tandem mass spectrometry (MS2) is a widely used method in structural biology in order to determine the sequence of biopolymers, such as proteins, DNAs, oligosaccharides... In MS2, an ion of interest is isolated, activated, and brought to dissociation. The analysis of the generated fragments provides structural information on the precursor ion. Recently, synchrotron radiation (SR) has been introduced as a new activation technique for MS2 [3].

In the present work, the potential of VUV-SR to provide structural information is probed through the study of a human IDP, namely IB5 [4]. The only described function of this salivary proline-rich protein (PRP) is to bind and scavenge tannins. However, the binding site of tannins on IB5 has never been precisely determined.

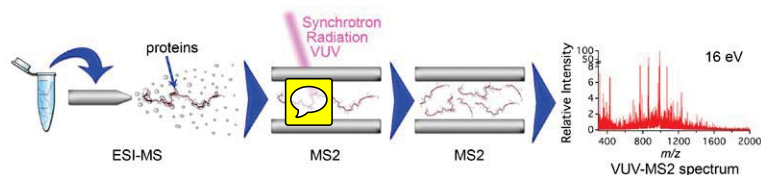
The coupling of an ion trap with the DESIRS beamline shows that SR provides an easy access to a wide variety of photon activations regimes, ranging from photodissociation (PD) to dissociative photoionization (DPI) (figure 1). The sequence coverage of IB5, obtained in DPI regime, was higher than with classical activation techniques (figure 2). It makes SR-MS2 an efficient sequencing method. Moreover, DPI has allowed for the first time to determine the binding site of the tannin, B2 3'OG, on IB5 (figure 3). The comparison between the IB5⁷⁺ and IB5•B2 3'OG⁷⁺ MS2 spectra obtained at 16 eV has allowed to identify and interpret more than fifty peaks as fragments of IB5 noncovalently bound to B2 3'OG. It appears that all these fragments from both N- and C-terminal series contain the KPQGPPPPQGG segment of the sequence, indicating a strong interaction between B2 3'OG and this part of the protein. This sequence with a cluster of five prolines very likely adopts a PPI or a PPIL helix conformation in solution. Such structural elements are thought to be crucial for IDPs in the binding with their partner, as this stable segment might provide an initial contact point. The role of the proline clusters in PRPs sequence, which were thought to be the tannin-binding site on PRP, is thus unequivocally confirmed. Therefore, this new method should open new perspectives in the growing and challenging fields of proteomics, structural biology and IDPs studies.



① Diagram of MS/MS technique based on activation by VUV synchrotron radiation.



② Nomenclature for MS/MS peptide fragmentation (a). Patterns of $IB5^{7+}$ protein fragments after activation at 6.4 and 16 eV by CID and ECD (c).



③ Localization of the B2 3'OG binding site on IB5. The objects $IB5^{7+}$ and $IB5 \cdot B2 \ 3'OG^{7+}$ were selected and irradiated with 16 eV photons (a). Comparison of the MS/MS 16 eV VUV spectra identified specific fragments in the $IB5 \cdot B2 \ 3'OG^{7+}$ fragmentation spectrum in which the m/z ratio had a mass difference corresponding to that of B2 3'OG $^{7+}$ compared with fragments from the fragmentation spectrum of $IB5^{7+}$ (b). CID activation of identified fragments confirmed the presence of the ligand (c). The map of B2 3'OG-carrying fragments identified the 'KPGQPPPPQGG' sequence as a B2 3'OG binding site on IB5.

DESIRS & DISCO beamlines

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Photodissociation and dissociative photoionization mass spectrometry of proteins and noncovalent protein–ligand complexes

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